CLAIMS

1. An *in vitro* method for measuring the activation status of neutrophil cells in a biological sample obtained from a mammal, preferably a horse, which method specifically measures the myeloperoxidase (MPO) content only, said content being correlated with said cell activation status, said method comprising the steps of:

- obtaining a biological sample, preferably a biological fluid from said mammal, preferably a horse, said sample containing said neutrophil cells and/or MPO released by said cells,
- (immuno)capturing MPO (1) present in said biological sample via a MPO-specific polyclonal or monoclonal antibody (2),
- detecting and/or measuring either total (active and inactive) MPO or exclusively active MPO present in said biological sample,
- possibly comparing the measured MPO values with normal MPO levels obtained from a significant number of healthy mammals, preferably horses,
- optionally quantifying MPO levels using a standard MPO curve, and
- possibly relating the MPO levels measured to an activity status of said cells indicative of the presence, absence or condition of a disease or immunological status.

2. The method according to claim 1 which is a sandwich ELISA detection method, wherein detection is obtained through a second labelled antibody (4) binding to a immunological complex formed by the first MPO-recognizing immobilized antibody (2) and the MPO (1), the method developed to detect and/or measure MPO, preferably equine MPO.

3. The method according to claim 1 which is a SIEFED detection method, wherein the step of immunocapturing MPO is followed by a washing step to remove any components that can interfere with the measurement of MPO enzymatic activity, the enzymatic activity of said myeloperoxidase (1) fixed on its specific antibody (2) then detected by adding a specific substrate to be transformed by the MPO (1) into a visible, preferably a fluorescent reaction product.

4. The method according to claim 3 wherein H_2O_2 and a suitable fluorimetric reaction product of a substrate, such as 10-acetyl-3,7-dihydroxyphenoxazine are added to the reaction medium, and preferably also a sufficient amount of nitrite to enhance a generated fluorescent signal.

5. The method according to any of claims 1 to 4 wherein said biological sample is a cellular or acellular sample selected from the group consisting of arterial, venous and capillary blood, serum, plasma, seminal fluid, broncho-alveolar fluid, urine, saliva, endotracheal fluid, peritoneal fluid, uterine irrigation liquids, sputum, synovial fluid, broncho-alveolar fluid, nasal fluid, gastric bowel and faecal derivate samples, cerebrospinal fluid and tissue extracts.

6. The method according to any of claims 1 to 5 wherein a neutrophil cell activation status is measured and possibly correlated to a disease and/or pathology.

7. An ELISA kit or device for measuring the activation status of neutrophil cells in a biological sample obtained from a mammal, preferably a horse, which ELISA kit or device specifically measures the total (active and inactive) myeloperoxidase (MPO) content, said content

being correlated with said cell activation status, said ELISA kit or device comprising the necessary elements for:

- immunocapturing MPO (1) that is present in a biological sample, preferably a biological fluid, obtained from a mammal, preferably a horse, and containing said neutrophil cells or MPO released by said cells, said immunocapturing being preferably obtained by a first MPO-recognizing polyclonal or monoclonal antibody (2) immobilized to a solid support (3),
- detecting and/or measuring active and inactive MPO present in said biological sample, preferably by a second enzymatically labelled MPO-recognizing polyclonal or monoclonal antibody (4) for detection of immunocaptured MPO (1),
- possibly comparing the measured MPO values with normal MPO levels obtained from a significant number of healthy mammals,
- optionally quantifying MPO levels using a standard MPO curve, and
- possibly relating the MPO levels measured to an activity status of said cells indicative of the presence, absence or condition of a disease or immunological status,

said detection and/or measurements specifically and accurately representing MPO levels in any type of biological sample.

8. A SIEFED kit or device for measuring the activation status of neutrophil cells in a biological sample obtained from a mammal, preferably a horse, which SIEFED kit or device specifically measures the active myeloperoxidase (MPO) content only, said content being correlated with said cell activation status, said SIEFED kit or device comprising the necessary elements for:

- immunocapturing MPO (1) present in a biological sample, preferably a biological fluid, obtained from a mammal, preferably a horse, the sample containing said neutrophil cells or MPO released by said cells,

- detecting and/or measuring active MPO present in said biological sample, whereby nitrite may be added to the reaction medium to amplify a myeoloperoxidase enzymatic reaction,

- possibly comparing the measured MPO values with normal MPO levels obtained from a significant number of healthy mammals, preferably horses,

- optionally quantifying MPO levels using a standard MPO curve, and

- possibly relating the MPO levels measured to an activity status of said cells indicative of the presence, absence or condition of a disease or immunological status,

said detection and/or measurements specifically and accurately representing active MPO levels in any type of biological sample.

9. The use of a method according to any of claims 1 to 6 or of a kit or device according to any of claims 7 to 8

- for the detection and/or prediction of a disease or pathology, preferably one selected from the group consisting of chronic or acute inflammatory diseases, digestive pathologies, strangulated intestinal pathologies, sepsis, septic shock, chronic and acute pulmonary pathologies (with invasion of the alveoli by neutrophils), ischemia-reperfusion pathologies, articular pathologies (with presence of neutrophils in the joints), colics, laminitis, allergies, infections and cardiovascular diseases,

- to follow-up neutrophil cell activation during therapy of a diseased mammal, preferably a horse,

- to evaluate the (natural) ability of neutrophil cells and/or drugs to fight against micro-organisms and/or to destroy them,

- to evaluate the efficiency of immunomodulators or the *in vitro* inhibitory capacity of drugs by comparing the neutrophil activation status of treated and non-treated neutrophils,

- to evaluate the ability of neutrophils treated with said modulators and/or drugs to against micro-organisms and/or to destroy them, or

- to evaluate the natural defense capacity or ability of a mammal, preferably a horse, to fight against micro-organisms.

- to the screen or to select compounds which interact with myeloperoxidase (MPO) and possibly inhibit myeloperoxidase (MPO) activity.

10. The use of nitrite, preferably a Na-salt thereof, to enhance enzymatic detection of a peroxidase, preferably to enhance a 10-acetyl-3,7-dihydroxyphenoxazine-induced fluorescence signal.

11. The use of both an ELISA and a SIEFED method to distinguish between total and active myeloperoxidase (MPO) content.

12. A screening and selection method of compound(s) interacting with myeloperoxidase (MPO), preferably inhibiting myeloperoxidase activity which comprises the step of

- capturing active MPO to a solid support,
- possibly measuring MPO activity,
- adding one or more compound(s) to the active MPO,
- measuring MPO activity after addition of the compound(s),

- possibly comparing MPO activities before or after addition of the compounds, and

- possibly recovering the compound(s) that interact with MPO, preferably compounds that inhibit myeloperoxidase activity.

13. The method of claim 12, wherein the step of capturing active MPO is done by an antibody, preferably a monoclonal antibody.

14. The method according to the claims 12 or 13, which comprises after the step of adding one or more compound(s) to the active MPO a step of washing of the compound(s) which are not bound to the active MPO.